Identification of multiple disease resistance in cowpea

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Between 1969 and 1975 the important diseases of cowpea in southern Nigeria were determined, methods to promote epidemics were studied and disease assessment scales were developed. The techniques developed were used to screen 5000 cowpea lines for field resistance to anthracnose, cercospora leaf spot, rust and bacterial pustule. After preliminary observations on 5000 lines, 719 lines were selected for further evaluation in field disease nurseries. All 719 lines were resistant to at least one of the four diseases, 685 lines were resistant to at least two, 537 lines resistant to at least three, 208 lines were resistant to all four and of these, 28 lines were also resistant to target spot. Preliminary yield evaluation indicated that some of these lines combined multiple resistance with moderate to high yield potential. In a separate study 16 of the multiple resistant lines were found resistant also to two isolates of cowpea (yellow) mosaic virus.

INTRODUCTION

The cowpea (*Vigna unguiculata* (L.) Walp.) is grown throughout the tropics for consumption of its leaves, green pods and grain. In several African countries, where more than 70 per cent of the world's cowpea crop is produced (FAO, 1972), the cowpea is the most important legume in the diet of the population, providing a major source of the protein intake of the rural and poor urban people.

The major areas of cowpea grain production are the savanna regions where yields are estimated to be about 0·4t/ha. However, grain yields of 1·5 to 2·0t/ha are readily attainable in experiment station trials (IITA, 1973, 1974) even in the forest region. The major constraints to increased on-farm cowpea production in the savanna region, and to widespread cultivation of cowpea in the forest region, are the severe effects of a large number of pests and diseases.

The Grain Legume Improvement Programme (*GLIP*) of the International Institute of Tropical Agriculture (IITA) has a major programme for the improvement of the cowpea in the lowland tropics. A major objective of

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the programme is to develop cowpea varieties of high yield potential with pest and disease resistance.

During 1971 to 1975 a large portion of the world cowpea germplasm collection was screened at IITA for susceptibility to the major diseases occurring in the region. This paper describes and discusses the methodology and results of the IITA cowpea screening programme for identification of resistance to the fungal and bacterial diseases. Another paper (WILLIAMS, 1977) discusses resistance to cowpea (yellow) mosaic virus.

MATERIALS AND METHODS

During the period 1969 to 1975 the IITA *GLIP* collected and classified more than 7000 cowpea lines from the tropics and sub-tropics (IITA, 1974). About 5000 of these lines were evaluated in this study.

Anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav.), cercospora leaf spot (*Cercospora cruenta* Sacc.), rust (*Uromyces vignae* Barcl.), bacterial pustule (*Xanthomonas* sp.), and cowpea (yellow) mosaic virus (*CPMV*) were identified as major diseases of cowpea in the region (WILLIAMS, 1975a). Target spot (*Corynespora cassiicola* (Berk & Curt) Wei), a second cercospora leaf spot (*Cercospora canescens* Ellis & Martin), and bacterial blight (*Xanthomonas vignicola* Burkholder) were regarded as relatively minor diseases (WILLIAMS, 1975a).

Screening philosophy and strategy

The large number of lines to be screened for susceptibility to several diseases, and the limited laboratory and glasshouse facilities, necessitated a field screening programme. The key requirements were (a) to expose the test lines evenly to inoculum under environmental conditions conducive to disease establishment and spread; (b) to use designs which allowed differences in degree of susceptibility to be observed; (c) to use disease severity assessment scales which reflected differences in the degree of susceptibility.

Between 1970 and 1972, techniques for managing and scoring the diseases and methodology for exposure of test lines to inoculum were developed.

Between 1971 and 1973 the first 3000 lines in the cowpea germplasm collection were grown for multiplication and general evaluation. During 1973 and 1974 an additional 2000 cowpea lines were grown and evaluated at IITA. Except in one planting no attempt was made to inoculate these lines, but natural disease occurrence was generally high. Disease incidence assessments were made in all plantings and during this process the scoring scales were developed, modified and eventually finalized. Those lines which showed little or no disease in the germplasm evaluation plantings were tested further for field resistance in a disease nursery programme.

Disease management and inoculum production

Cowpea lines consistently developing severe incidence of the major fungal and bacterial diseases were maintained in 'disease gardens' throughout

the year. Overhead irrigation was used to establish the disease gardens and promote disease development when rainfall was inadequate.

Cercospora leaf spot (*GLS*) and rust were easily established by scattering infected leaves from an older disease garden among the susceptible plants (TVu 57 for *CLS*; TVu 2331 and TVu 76 for rust) about 28 days after planting (*DAP*). *CLS* built up rapidly at all times of the year, but rust development was slower during periods of frequent heavy rains.

Bacterial pustule was established by spraying 28-day-old plants (TVu 880 and TVu 91) with suspensions derived from macerated infected leaves which were either freshly picked or maintained in a deep freeze. Irrigation before inoculation and inoculation towards evening ensured effective take. Bacterial pustule build-up and spread was most rapid during periods of frequent heavy rains.

Anthracnose was established by scattering heavily infected stem pieces, freshly cut from an older disease garden, amongst the 28-day-old susceptible plants (TVu 57 and TVu 91) during frequent rains or overhead irrigation. Anthracnose build-up was most rapid during cool rainy weather.

Target spot was not artificially promoted, but developed extensively in all plantings as the plants neared maturity.

Disease assessment scales

The following disease assessment scales were developed for grading the reactions of the test lines to the fungal and bacterial diseases in the disease nurseries.

A. Scale for recording anthracnose reactions
1. No symptoms, or flecking of stem from hypersensitive reactions
2. Scattered, few, small narrow lestons
3. Scattered, few, large lesions
4. Many small narrow lesions
5. Many large lesions
6. Large parts of stems with coalesced lesions
7. Most of the plant, stems, petioles, peduncles, leaf veins covered with coalesced lesions
8. Line obviously segregating

B. Scale for recording *CLS* and target spot reactions
1. No symptoms
2. Occasional, scattered leaf spots
3. Scattered spots, no more than one per leaf, on more than half the leaves
4. Two or three spots per leaf on few leaves
5. Two or three spots per leaf on most of the leaves
6. Many spots on a few leaves
7. Many spots on most leaves with yellowing and defoliation occurring
8. Line obviously segregating

C. Scale for recording rust and bacterial pustule reactions
1. No symptoms
2. Scattered pustules on less than half of the leaves
3. Scattered pustules on most leaves

4. Small areas (up to 25 per cent) of leaf surface with large concentration of pustules, on less than half of the leaves
5. Small areas (up to 25 per cent) of leaf surface with large concentrations of pustules on most of the leaves
6. Most of the leaf surface of less than half of the leaves covered in large pustule concentrations
7. Most of the leaf surface of most leaves covered with large pustule concentrations and leaf yellowing and defoliation occurring
8. Line obviously segregating

Design and operation of the disease nurseries

In the Preliminary Disease Nurseries (*PDN*) the test lines were planted in single 3 m rows, with single seeds planted every 10 cm, with 1·5 m between rows. At both ends of the test rows, outside the row marker pegs, four seeds of each four known susceptible 'spreader' lines were planted about 14 days prior to planting the test rows. Planting was timed so that the test rows were subjected to consistent heavy rainfall when they were between four and eight weeks old. The spreaders were inoculated as described above about 28 *DAP*. Disease incidence was recorded in the test lines between 50 and 57 *DAP*. Known susceptible lines were included as test lines to check that the inoculation procedure and the environment were conducive for rapid disease build-up. Lines showing little or no development of three or more diseases in the *PDN* were further tested in the Advanced Disease Nurseries (*ADN*).

The *ADN* were managed in the same way as the *PDN*. Test lines were represented in plots of two 4 m rows 50 cm apart, with two replications. In addition to disease assessments, yield assessments were made of the central 3 m^2 of each plot, using a 3 m × 1 m metal quadrat and permanent corner markers of the harvest area to allow multiple pickings.

RESULTS

A total of 719 lines were tested in *PDN* and 204 lines were tested in at least one *ADN*. The reaction of the 719 lines to each of the five diseases indicates the availability of a large pool of resistance and low susceptibility (*Table 1*).

Table 1. Number of cowpea lines with various scores to five diseases in disease nurseries

	Disease index†							
*Disease**	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*‡
Anthracnose	303	112	15	31	18	54	11	175
Cercospora leaf spot	562	24	10	12	15	14	36	46
Rust	624	32	1	5	2	20	18	17
Bacterial pustule	556	10	4	46	16	41	18	28
Target spot	324	273	13	43	22	25	19	0

* Except for target spot, inoculum was provided by highly susceptible lines planted as 'spreaders' throughout the nurseries
† 1 = Freedom from the disease, 7 = Severe disease
‡ Lines obviously segregating or mixed

Table 2. Number of cowpea lines with resistance to two or more of five diseases in disease nurseries

*Definition**	*Number of lines*
Free from Anth, CLS, rust, BP and TS	28
Free from Anth, CLS, rust and BP	128
Free/slight Anth, free from CLS, rust and BP	208
Free/slight Anth, free from CLS and rust	278
Free/slight Anth, free from CLS and BP	253
Free/slight Anth, free from rust and BP	257
Free from CLS, rust and BP	373
Free/slight Anth, free from CLS	327
Free/slight Anth, free from rust	337
Free/slight Anth, free from BP	319

* Anth = anthracnose; CLS = *Cercospora cruenta* leaf spot; BP = bacterial pustule; TS = target spot

Table 3. Origins and pedigrees of 28 cowpea lines with combined resistance to anthracnose, cercospora leaf spot, rust, bacterial pustule and target spot at Ibadan, Nigeria*

Line (TVu No.)	*Pedigree*	*Origin*	*Line (TVu No.)*	*Pedegree*	*Origin*
8–1	C–1 Sel	Nigeria	3273	PI 354494	India
62–2	Local White × Dixielee selection	Nigeria	3408	PI 354658	India
			3415	PI 354667	India
64–1	Dixielee O/C selection	Nigeria	3430–1	PI 354806	India
176	C 5723–11	U.S.A.	3511	PI 354806	India
201–1D	Outcross selection	Nigeria	3521	PI 354821	India
317	C 5810–6	U.S.A.	3552	PI 354857	India
374–1	C 5711–1–2–selection	U.S.A.	3847–1	KR 194 selection	Nigeria
1190	V.U.5 selection	Kenya			
2657	PI 339593	South Africa	4536	IF H79–2	Nigeria
2757	No. 10	India	4546	IF H1 O/C–1	Nigeria
2785	No. 46	India	4549	IF H27–8	Nigeria
2846	PI 353000	India	4557	IF H53–1	Nigeria
2847	PI 353094 selection	India	4558	IF H82–1	Nigeria
2939–1	PI 355094 selection	India	4562	IF H82–1	Nigeria

* Lines with numerals separated by a dash, e.g. 374–1, are selections made at IITA from the original line

Table 4. Summary of yield performance, growth habit and disease reaction of eight cowpea lines

Line	*Resistant to**	*Growth habit*	*Grain yield at 14% moisture (kg/ha)*	*Amount (%) harvested at*		
				63 DAP	*73 DAP*	*83 DAP*
2616P–02D	CLS, BP, R, A	Semi-erect	1740	56	38	6
347	CLS, BP, R, A, CV	Intermediate	1698	0	72	28
1595	CLS, BP, R, A	Intermediate	1684	24	51	25
2004	BP, R, A, CY	Intermediate	1656	45	51	4
1485P–2	CLS, BP, R, A	Intermediate	1588	37	47	16
1065	CLS, BP, R, A	Semi-erect	1562	0	84	16
853	CLS, BP, R, A	Intermediate	1549	32	54	14
201–1D	CLS, BP, R, A, CV, CY	Intermediate	1544	41	53	6

* CLS = *Cercospora cruenta* leaf spot; BP = bacterial pustule; R = rust; A = anthracnose; CV = cowpea mosaic virus; CY = corynespora leaf spot
DAP denotes days after planting

Lines with a disease index of three or less were considered the most useful sources of resistance. Further analysis of the disease incidence data reveals the occurrence of considerable multiple resistance within the 719 lines, with 28 lines free of all five diseases, 128 lines free of the four major diseases and 208 lines free of or highly resistant to the four major diseases (*Table 2*). There were 685 lines resistant to at least two of the four major diseases, and 537 lines resistant to at least three of the four major diseases. The 28 lines free from the five diseases came mainly from Nigeria and India (*Table 3*). However, there was apparently some duplication of lines and genetic studies will be required to determine the number of different resistant genotypes.

Of the 204 lines given a preliminary yield evaluation in the *ADN*, 55 produced grain at the rate of more than 200 g/m^2. Twenty five of these were further evaluated in larger plots (9 m^2) during the September–November growing season 1974. All 25 yielded more than 1 t/ha and eight yielded in excess of 1·5 t/ha (*Table 4*).

DISCUSSION

In this study methods were developed which enabled the assessment of the field reactions of several hundred cowpea lines to exposure to inoculum of fungal and bacterial pathogens. The key factors in the success of the study were the ability to promote development of the diseases when required and the availability of a large diverse germplasm collection. For the identification of lines with field resistance the disease nursery system used in this study is considered preferable to laboratory or glasshouse testing in which the number of lines that can be handled is limited, plant growth and reactions in the high temperature–low light intensity environment are abnormal and adult plant reactions are not normally obtained.

The study has identified many élite sources of resistance to the bacterial and fungal diseases combined with moderate to high yield potential. In addition, subsequent tests have shown 16 of the multiple resistant lines to be resistant to two isolates of cowpea (yellow) mosaic virus (WILLIAMS, 1977).

This study represents the beginning of the process of identifying sources of stable disease resistance. The lines identified in this study should be tested at several different locations around the world during several seasons so that they are exposed to different populations of the various pathogens under a range of environmental conditions. In this way sources of broad-spectrum stable resistance can be identified. Such a multilocational programme was initiated in 1974 with 100 lines tested against various pathogens in Puerto Rico, Nigeria and India (WILLIAMS, 1975b). Many additional locations are required to increase the range of variability of pathogens and pathogen populations. Locations are required where epiphytotics of septoria leaf spot, synchytrium false rust, powdery mildew and fusarium wilt occur regularly, and where the pathogens causing rust, anthracnose, cercospora leaf spot and bacterial pustule may have evolved under different selection pressures to produce different pathotypes.

Studies are needed to determine the inheritance of the identified resistance. This information will enable the intelligent choice of lines for hybridization

programmes and will allow predictions on the probability of resistance stability. The IITA *GLIP* will be pleased to supply seed of cowpea lines to individuals or organizations for such studies on a cooperative basis.

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